PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 25 May 2000 (25.05.00)	
International application No. PCT/CA99/00917	**Applicant's or agent's file reference** 1038-984 MIS
International filing date (day/month/year) 04 October 1999 (04.10.99)	**Priority date** (day/month/year) 05 October 1998 (05.10.98)
Applicant PARRINGTON, Mark et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

03 May 2000 (03.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Claudio Borton
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 b low.
1038-984 MIS		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/CA 99/ 00917	04/10/1999	05/10/1998
Applicant		
CONNAUGHT LABORATORIES LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] it is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [X] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readbie form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rul 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 99/00917

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 561 058 A (SIGUA CHRISTOPHER L ET AL) 1 October 1996 (1996-10-01) column 1-10 column 19, paragraph 3 column 26 examples 1,5,6,10 ---	1-14
X	HABERHAUSEN ET AL: "Comparative study of different standardization concepts in quantitative competitive reverse transcription PCR-assays" JOURNAL OF CLINICAL MICROBIOLOGY, vol. 36, no. 3, March 1998 (1998-03), pages 628-633, XP002132721 the whole document --- -/--	1-14

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 March 2000	02/05/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Reuter, U

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TRABAUD ET AL: "Development of a reverse transcriptase PCR-enzyme-linked immunosorbent assay for quantification of human immunodeficiency virus type 1 RNA in plasma: comparison with commercial quantitative assays" JOURNAL OF CLINICAL MICROBIOLOGY, vol. 35, no. 5, May 1997 (1997-05), pages 1251-1254, XP002132722 the whole document	1-14
Y	ROBINSON M O ET AL: "DETERMINING TRANSCRIPT NUMBER USING THE POLYMERASE CHAIN REACTION: PGK-2, MP2, AND PGK-2 TRANSGENE MRNA LEVELS DURING SPERMATOGENESIS" NUCLEIC ACIDS RESEARCH,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 19, no. 7, 11 April 1991 (1991-04-11), pages 1557-1562, XP000303861 ISSN: 0305-1048 the whole document	1-14
Y	HEID C A ET AL: "REAL TIME QUANTITATIVE PCR" GENOME RESEARCH,US,COLD SPRING HARBOR LABORATORY PRESS, vol. 6, no. 10, 1 October 1996 (1996-10-01), pages 986-994, XP000642795 ISSN: 1088-9051 the whole document	1-14
A	HOOF T ET AL: "QUANTITATION OF MRNA BY THE KINETIC POLYMERASE CHAIN REACTION ASSAY: A TOOL FOR MONITORING P-GLYCOPROTEIN GENE EXPRESSION" ANALYTICAL BIOCHEMISTRY,US,ACADEMIC PRESS, SAN DIEGO, CA, vol. 196, no. 1, 1 July 1991 (1991-07-01), pages 161-169, XP000231207 ISSN: 0003-2697 the whole document	1-14
A	WO 97 41261 A (IMMUNOLOGICAL ASSOCIATES OF DE) 6 November 1997 (1997-11-06) the whole document	1-14

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 99/00917

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5561058 A	01-10-1996	US 5693517 A	02-12-1997
		US 5310652 A	10-05-1994
		US 5418149 A	23-05-1995
		US 5322770 A	21-06-1994
		US 4889818 A	26-12-1989
		AU 675318 B	30-01-1997
		AU 6594694 A	12-01-1995
		CA 2127188 A	02-01-1995
		EP 0632134 A	04-01-1995
		JP 7059599 A	07-03-1997
		US 5618703 A	08-04-1997
		US 5641864 A	24-06-1997
		US 5618711 A	08-04-1997
		US 5789224 A	04-08-1998
		AT 176002 T	15-02-1999
		AU 665338 B	04-01-1996
		AU 8532791 A	18-02-1992
		CA 2087724 A	25-01-1992
		DE 69130800 D	04-03-1999
		DE 69130800 T	16-09-1999
		EP 0540693 A	12-05-1993
		ES 2128323 T	16-05-1999
		GR 3029987 T	30-07-1999
		JP 6501612 T	24-02-1994
		WO 9201814 A	06-02-1992
		US 5795762 A	18-08-1995
		US 5466591 A	14-11-1995
		AT 151112 T	15-04-1997
		AU 656315 B	02-02-1995
		AU 7244491 A	24-07-1991
		CA 2071213 A	23-06-1991
		DE 69030386 D	07-05-1997
		DE 69030386 T	09-10-1997
		DK 506889 T	22-09-1997
		EP 0506889 A	07-10-1992
		ES 2100945 T	01-07-1997
		GR 3023862 T	30-09-1997
		JP 2968585 B	25-10-1999
		JP 5505105 T	05-08-1993
		US 5407800 A	18-04-1995
		WO 9109944 A	11-07-1991
		AT 169337 T	15-08-1998
		AU 681387 B	28-08-1997
		AU 6329694 A	01-09-1994
		AU 646430 B	24-02-1994
		AU 7176491 A	24-07-1991
		CA 2071196 A	23-06-1991
		DE 69032543 D	10-09-1998
		DE 69032543 T	15-04-1999
		EP 0506825 A	07-10-1992
WO 9741261 A	06-11-1997	AU 3116997 A	19-11-1997
		EP 0910666 A	28-04-1999

PATENT COOPERATION

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

STEWART, M
Sim & McBurney
330 University Avenue
6th floor
Toronto, Ontario M5G 1R7
CANADA

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	08.11.2000
Applicant's or agent's file reference 1038-984 MIS	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA99/00917	04/10/1999	05/10/1998

Applicant
CONNAUGHT LABORATORIES LIMITED et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Danti, B Tel.+49 89 2399-8161

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
1038-984 MIS		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA99/00917	04/10/1999	05/10/1998
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant CONNAUGHT LABORATORIES LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which hav been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03/05/2000	08.11.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer BROCHADO GARGANTA, M Telephone No. +49 89 2399 8935

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA99/00917

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-18 as originally filed

Claims, No.:

1-14 as originally filed

Drawings, sheets:

1/8-8/8 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-14
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-14
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

   (A) US-A-5 561 058

   (B) Haberhausen et al.: Journal of Clinical Microbiology, vol. 36, no. 3, (1998-03), pages 628-633

   (C) Trabaud et al.: Journal of Clinical Microbiology, vol. 35, no. 5, (1997-05), pages 1251-1254

2.1 The subject-matter of claim 1, referring to a method for determining the quantity of a target RNA in a tissue sample, is not new in the sense of Article 33(2) PCT, because such a method is already disclosed in documents A, B and C.

Document A discloses a method for amplifying a target RNA molecule in a sample (see claim 1). Total RNA is isolated from cells, for example from blood samples (see column 8, lines 51-65 and column 39, lines 60-67). The isolated RNA is reverse transcribed and amplified, using primers corresponding to transcribed sequences of the target RNA (see example VI). The detection of reverse transcribed or amplified products is accomplished by hybridisation with isotopic or non-isotopically labelled probes in for example a dot-blot or electrophoretic format (see column 19, lines 47-62). The amount of bound labelled sequence in the sample is compared with the RNA standard as a measure of the number of copies of target RNA in the sample (see examples VI and VII).

Document B discloses a comparative study of different standardisation concepts in quantitative reverse transcription -PCR assays, wherein plasma samples are isolated from patients with hepatitis (see title and page 628, column 2). Total RNA is isolated from plasma cells and subjected to reverse transcription and DNA amplification, using primers corresponding to the transcribed sequences of the target RNA (see Materials

and Methods). The detection of the amplified material is done using a nonisotopic approach based on electrochemiluminiscence with a ruthenium-tris(bipyridyl)-labelled oligonucleotide and hybridisation to the biotinylated denatured amplicom (see page 629, Materials and Methods). Statistics were calculated based on sample measurements, allowing a direct comparison of different procedures after the establishment of standard curves (see page 629 and table I).

Document C discloses a reverse transcriptase PCR-enzyme-linked immunosorbent assay for quantification of human immunodefficiency virus in plasma (see title). RNA is extracted from plasma from patients, reverse transcribed and amplified using the incorporation of digoxigenin-labelled dUTP during amplification and detection of labelled PCR products by hybridisation with biotinylated probes. The coamplification of an internal standard RNA and the wild-type RNA of the sample allows the establishment of a standard curve and thereby the calculation of the RNA copy number in the sample (see page 1251, column 1).

2.2 For the same reasons, the features of dependent claims 2-11 are also not novel in the sense of Article 33(2) PCT. The features of claims 2-3 are disclosed in document C (see pages 1251-1252). The features of claims 4-11 are known from document A (see column 3, lines 57-61; example 8; column 10, lines 1-3; column 19, lines 47-62; and claims 1 and 9).

2.3 Claim 12 discloses a method for determining the quantity of a target RNA in a tissue sample, with the same features of the method disclosed in claim 1, with the difference that the labelled sequence will hybridise with the PCR product and not with the RNA. This feature is already disclosed in documents A (see table 1 and column 30), B (see Materials and Methods) and C (page 1251). Thus, the subject-matter of claim 12 is not new (Article 33(2) PCT).

2.4 The subject-matter of claim 13, referring to a method for quantifying more accurately a target RNA in a tissue sample, is also not novel according to Article 33(2) PCT (see documents A, example I; document B, RT-PCR assays ; document C, page 1251).

The additional features of dependent claim 14 are also known from documents A, B

and C (see respectively: document A, column 19; document B, detection protocol, page 629; document C, page 1251, column 1). Thus, claim 14 is also not new (Article 33(2) PCT).

Re Item VIII
Certain observations on the international application

1. The independent claims (claims 1, 12 and 3) refer to a similar method only varying in the labelled sequence, that can correspond to the internal transcribed sequence of the target RNA, be complementary to one of the strands of the PCR product of the target RNA or correspond to an internal sequence of the amplified product.

2. In claim 2, the wording "to provide a plurality of samples of known starting copy number" refers to the result to be achieved by the serial dilution and does not characterise the method (see Guidelines III-4.7).

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
1038-984 MIS		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA99/00917	04/10/1999	05/10/1998
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant CONNAUGHT LABORATORIES LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Auth rity and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03/05/2000	08.11.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer BROCHADO GARGANTA, M Telephone No. +49 89 2399 8935

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA99/00917

I. Basis f the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-18 as originally filed

Claims, No.:

1-14 as originally filed

Drawings, sheets:

1/8-8/8 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they hav been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA99/00917

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-14
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-14
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

   (A) US-A-5 561 058

   (B) Haberhausen et al.: Journal of Clinical Microbiology, vol. 36, no. 3, (1998-03), pages 628-633

   (C) Trabaud et al.: Journal of Clinical Microbiology, vol. 35, no. 5, (1997-05), pages 1251-1254

2.1 The subject-matter of claim 1, referring to a method for determining the quantity of a target RNA in a tissue sample, is not new in the sense of Article 33(2) PCT, because such a method is already disclosed in documents A, B and C.

Document A discloses a method for amplifying a target RNA molecule in a sample (see claim 1). Total RNA is isolated from cells, for example from blood samples (see column 8, lines 51-65 and column 39, lines 60-67). The isolated RNA is reverse transcribed and amplified, using primers corresponding to transcribed sequences of the target RNA (see example VI). The detection of reverse transcribed or amplified products is accomplished by hybridisation with isotopic or non-isotopically labelled probes in for example a dot-blot or electrophoretic format (see column 19, lines 47-62). The amount of bound labelled sequence in the sample is compared with the RNA standard as a measure of the number of copies of target RNA in the sample (see examples VI and VII).

Document B discloses a comparative study of different standardisation concepts in quantitative reverse transcription -PCR assays, wherein plasma samples are isolated from patients with hepatitis (see title and page 628, column 2). Total RNA is isolated from plasma cells and subjected to reverse transcription and DNA amplification, using primers corresponding to the transcribed sequences of the target RNA (see Materials

and Methods). The detection of the amplified material is done using a nonisotopic approach based on electrochemiluminiscence with a ruthenium-tris(bipyridyl)-labelled oligonucleotide and hybridisation to the biotinylated denatured amplicom (see page 629, Materials and Methods). Statistics were calculated based on sample measurements, allowing a direct comparison of different procedures after the establishment of standard curves (see page 629 and table I).

Document C discloses a reverse transcriptase PCR-enzyme-linked immunosorbent assay for quantification of human immunodefficiency virus in plasma (see title). RNA is extracted from plasma from patients, reverse transcribed and amplified using the incorporation of digoxigenin-labelled dUTP during amplification and detection of labelled PCR products by hybridisation with biotinylated probes. The coamplification of an internal standard RNA and the wild-type RNA of the sample allows the establishment of a standard curve and thereby the calculation of the RNA copy number in the sample (see page 1251, column 1).

2.2 For the same reasons, the features of dependent claims 2-11 are also not novel in the sense of Article 33(2) PCT. The features of claims 2-3 are disclosed in document C (see pages 1251-1252). The features of claims 4-11 are known from document A (see column 3, lines 57-61; example 8; column 10, lines 1-3; column 19, lines 47-62; and claims 1 and 9).

2.3 Claim 12 discloses a method for determining the quantity of a target RNA in a tissue sample, with the same features of the method disclosed in claim 1, with the difference that the labelled sequence will hybridise with the PCR product and not with the RNA. This feature is already disclosed in documents A (see table 1 and column 30), B (see Materials and Methods) and C (page 1251). Thus, the subject-matter of claim 12 is not new (Article 33(2) PCT).

2.4 The subject-matter of claim 13, referring to a method for quantifying more accurately a target RNA in a tissue sample, is also not novel according to Article 33(2) PCT (see documents A, example I; document B, RT-PCR assays ; document C, page 1251).

The additional features of dependent claim 14 are also known from documents A, B

and C (see respectively: document A, column 19; document B, detection protocol, page 629; document C, page 1251, column 1). Thus, claim 14 is also not new (Article 33(2) PCT).

Re Item VIII
Certain observations on the international application

1. The independent claims (claims 1, 12 and 3) refer to a similar method only varying in the labelled sequence, that can correspond to the internal transcribed sequence of the target RNA, be complementary to one of the strands of the PCR product of the target RNA or correspond to an internal sequence of the amplified product.

2. In claim 2, the wording "to provide a plurality of samples of known starting copy number" refers to the result to be achieved by the serial dilution and does not characterise the method (see Guidelines III-4.7).

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12Q 1/68 — A3

(11) International Publication Number: **WO 00/20629**

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(57) Abstract

An accurate method of determining the quantity of specific RNA in a tissue sample permits analysis of rare transcripts, such as cytokines, and is based on a modified RNA isolation procedure, RT–PCR in a single enzyme reaction, detection and quantification, preferably employing an RNA standard.

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INTERNATIONAL SEARCH REPORT

onal Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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[X] Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search	Date of mailing of the international search report
10 March 2000	02/05/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Reuter, U

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